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CRISPR-Cas Genome Editing Technology: Applications, Advances and Challenges in Crop Stress Resistance Breeding

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ABSTRACT
The dual challenges of climate change and population growth have intensified biotic and abiotic stresses on crops, threatening global food security. CRISPR-Cas genome editing technology, as a revolutionary tool in agricultural biotechnology, has been widely applied in crop breeding due to its high precision, efficiency and simplicity. This review systematically summarizes the applications of CRISPR-Cas technology in crop resistance breeding against abiotic stresses (drought, salinity, extreme temperature) and biotic stresses (pathogens, pests). It also highlights the latest advances in CRISPR-Cas systems (e.g., base editing, prime editing) and their optimization strategies for crop improvement. Additionally, the potential challenges (off-target effects, regulatory policies, public acceptance) and future prospects of CRISPR-Cas technology in agricultural production are discussed. This review provides a theoretical basis and technical reference for the application of genome editing technology in sustainable crop breeding.

Keywords: CRISPR-Cas technology; genome editing; crop breeding; stress resistance; abiotic stress; biotic stress; agricultural biotechnology

1. Introduction

Global food security is facing unprecedented challenges driven by rapid population growth, climate change, and environmental degradation. It is estimated that the global population will reach 9.7 billion by 2050, requiring a 70% increase in crop production to meet the growing demand for food (FAO, 2023). Meanwhile, climate change-induced abiotic stresses (such as drought, salinity, extreme temperature) and biotic stresses (including fungal, bacterial, viral pathogens and insect pests) have caused significant crop yield losses, accounting for 30%-50% of global crop production annually (Riaz et al., 2025). Traditional crop breeding methods, such as cross-breeding and mutagenesis, have made important contributions to crop improvement, but they are limited by long breeding cycles, low efficiency, and difficulty in precise trait improvement (Zhang et al., 2024).

The development of agricultural biotechnology has brought new opportunities for crop breeding. Following the development of transgenic technology, genome editing technology has emerged as a powerful tool for precise genetic modification of crops. Among various genome editing technologies (such as ZFNs, TALENs), the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins) system has attracted widespread attention due to its simplicity, high efficiency, low cost, and wide

applicability (Movahedi & Yang, 2025). Since its first application in plant genome editing in 2013, CRISPR-Cas technology has been rapidly applied to various crops, including major food crops (rice, wheat, maize) and cash crops (cotton, soybean, tomato), achieving significant progress in improving crop stress resistance, yield, and quality (Wang et al., 2023).

The Agro-Biotechnolog journal focuses on the basic and applied research of agricultural biotechnology, covering plant biotechnology, microbial biotechnology, food science, and other related fields (Unisza, 2023). In line with the journal's scope, this review focuses on the applications of CRISPR-Cas genome editing technology in crop stress resistance breeding. We systematically summarize the application progress of CRISPR-Cas technology in improving crop resistance to abiotic and biotic stresses, introduce the latest advances in CRISPR-Cas systems and their optimization strategies, analyze the existing challenges, and discuss future development prospects. This review aims to provide a comprehensive reference for researchers engaged in crop breeding and agricultural biotechnology, and promote the application of CRISPR-Cas technology in sustainable agricultural development.

2. Overview of CRISPR-Cas Genome Editing Technology

2.1 Basic Structure and Working Principle of CRISPR-Cas System

The CRISPR-Cas system is an adaptive immune system evolved by bacteria and archaea to resist the invasion of phages and foreign plasmids (Barrangou et al., 2007). It consists of two core components: the CRISPR locus and the Cas protein gene cluster. The CRISPR locus is composed of a series of short palindromic repeat sequences (20-30 bp) separated by spacer sequences (20-30 bp), which are derived from the genome of invading phages or plasmids. The Cas protein gene cluster is located upstream or downstream of the CRISPR locus, encoding a series of Cas proteins with nuclease, helicase, or other functions (Makarova et al., 2023).

The working principle of the CRISPR-Cas system can be divided into three stages: adaptation, expression, and interference. In the adaptation stage, when phages or foreign plasmids invade, bacteria capture the foreign DNA fragments and integrate them into the CRISPR locus as new spacer sequences, forming a „memory" of the invader. In the expression stage, the CRISPR locus is transcribed into a long precursor CRISPR RNA (pre-crRNA), which is processed into mature crRNA under the action of Cas proteins and other auxiliary proteins. The mature crRNA forms a ribonucleoprotein complex (RNP) with the Cas protein. In the interference stage, the crRNA in the RNP complex recognizes and binds to the complementary foreign DNA sequence (protospacer) through base pairing, and the Cas protein cleaves the target DNA, thereby inhibiting the replication and expression of foreign genetic material (Zhang et al., 2023).

Based on the structure and function of Cas proteins, the CRISPR-Cas system can be divided into two classes and six types. Class 1 includes types I, III, and IV, which rely on multiple Cas proteins to form a complex to cleave target DNA. Class 2 includes types II, V, and VI, which rely on a single Cas protein (such as Cas9, Cas12a, Cas13a) to complete the cleavage reaction. Among them, the type II CRISPR-Cas9 system is the most widely used in crop genome editing due to its simple structure and easy operation (Movahedi & Yang, 2025). The Cas9 protein has two nuclease domains (HNH and RuvC), which can cleave the two strands of target DNA respectively, generating double-strand breaks (DSBs) at the target site. The DSBs can be repaired by two main pathways in plant cells: non-homologous end joining (NHEJ) and homologous recombination (HR). The NHEJ pathway is error-prone, often causing small insertions or deletions (indels) at the cleavage site, leading to gene knockout. The HR pathway is relatively accurate, which can introduce specific genetic

modifications (such as gene insertion or replacement) with the help of a homologous template (Wang et al., 2024).

2.2 Advantages of CRISPR-Cas Technology Compared with Traditional Breeding and Transgenic Technology

Compared with traditional crop breeding methods, CRISPR-Cas genome editing technology has obvious advantages. First, it has high precision. Traditional cross-breeding often involves the transfer of multiple genes, leading to linkage drag, while CRISPR-Cas technology can target specific genes for modification, avoiding the interference of other genes. Second, it has high efficiency. The breeding cycle of traditional cross-breeding is usually 5-10 years, while CRISPR-Cas technology can complete the modification of target traits in 1-2 generations, greatly shortening the breeding cycle. Third, it has wide applicability. CRISPR-Cas technology can be applied to various crops, including monocotyledonous crops (rice, wheat, maize) and dicotyledonous crops (cotton, soybean, tomato), and can modify multiple genes simultaneously (multiplex editing) (Li et al., 2023).

Compared with transgenic technology, CRISPR-Cas technology also has unique advantages. Transgenic technology usually involves the introduction of foreign genes into crop genomes, which may cause public concerns about food safety and environmental risks. In contrast, CRISPR-Cas technology can modify the endogenous genes of crops without introducing foreign genes, generating gene-edited crops that are similar to those obtained by traditional mutagenesis breeding. Therefore, gene-edited crops are more likely to be accepted by the public and pass regulatory reviews (Lubie Nie Chi et al., 2025). In addition, CRISPR-Cas technology is simpler and cheaper to operate than transgenic technology, which is more suitable for large-scale application in crop breeding.

2.3 Optimization of CRISPR-Cas Technology in Crop Editing

Although the CRISPR-Cas system has been widely applied in crop genome editing, there are still some problems that need to be solved, such as low editing efficiency, off-target effects, and difficulty in delivering editing tools into plant cells. In recent years, researchers have made significant progress in optimizing the CRISPR-Cas system to improve its application effect in crop breeding.

In terms of improving editing efficiency, various strategies have been developed. First, optimizing the sgRNA (single guide RNA) design. The sgRNA is a chimeric RNA composed of crRNA and tracrRNA, which is responsible for guiding the Cas9 protein to the target site. The efficiency of sgRNA is closely related to its sequence and structure. By optimizing the length of sgRNA, the GC content, and the distance from the PAM (Protospacer Adjacent Motif) sequence, the binding efficiency of sgRNA to the target site can be improved, thereby enhancing editing efficiency (Zhang et al., 2024). Second, using Cas protein variants. Researchers have developed a variety of Cas9 variants with higher editing efficiency, such as Cas9-HF1, eSpCas9, and SpCas9-NG. These variants have improved the specificity and efficiency of DNA cleavage, and expanded the range of target sites (Movahedi & Yang, 2025). Third, optimizing the delivery method of editing tools. The delivery of CRISPR-Cas editing tools into plant cells is a key step in genome editing. Common delivery methods include Agrobacterium-mediated transformation, particle bombardment, and protoplast transfection. By optimizing the delivery vector, the concentration of editing tools, and the transformation conditions, the delivery efficiency can be improved, thereby enhancing editing efficiency (Li et al., 2023).

In terms of reducing off-target effects, several effective strategies have been proposed. Off-target effects refer to the cleavage of non-target DNA sequences by the CRISPR-Cas system, which may lead to unexpected

genetic mutations and affect crop traits. First, improving the specificity of sgRNA. By designing sgRNA with high specificity and avoiding homologous sequences in the crop genome, the occurrence of off-target effects can be reduced. Second, using high-specificity Cas protein variants. Cas9 variants such as Cas9-HF1 and eSpCas9 have reduced off-target cleavage activity while maintaining high on-target editing efficiency (Wang et al., 2024). Third, using double-nicking strategy. The double-nicking strategy uses two sgRNAs to guide the Cas9 nickase (Cas9n) to cleave the two strands of target DNA respectively, generating a DSB only at the target site, which can significantly reduce off-target effects (Movahedi & Yang, 2025). Fourth, detecting and evaluating off-target effects. Various methods have been developed to detect off-target effects, such as whole-genome sequencing (WGS), targeted deep sequencing, and GUIDE-seq. By detecting off-target sites and evaluating their effects, the safety of gene-edited crops can be ensured (Zhang et al., 2023).

3. Applications of CRISPR-Cas Technology in Crop Abiotic Stress Resistance Breeding

Abiotic stresses, including drought, salinity, extreme temperature (high temperature and low temperature), and heavy metal stress, are major factors limiting crop growth and yield. CRISPR-Cas genome editing technology has been widely applied in improving crop resistance to various abiotic stresses, achieving significant progress. This section summarizes the application of CRISPR-Cas technology in crop drought resistance, salt resistance, and extreme temperature resistance breeding.

3.1 Drought Resistance Breeding

Drought is one of the most serious abiotic stresses, which can cause crop wilting, photosynthesis inhibition, and yield reduction. Improving crop drought resistance is crucial for ensuring food security in arid and semi-arid regions. CRISPR-Cas technology has been used to edit drought-responsive genes in various crops, improving their drought resistance.

Rice is one of the most important food crops in the world, and drought stress has a significant impact on its yield. Researchers have used CRISPR-Cas9 technology to edit multiple drought-responsive genes in rice, improving its drought resistance. For example, the OsDREB2A gene is a key transcription factor involved in rice drought stress response, which can regulate the expression of downstream drought-resistant genes. By overexpressing OsDREB2A using CRISPR-Cas9-mediated gene activation technology, researchers obtained rice lines with significantly improved drought resistance, which showed higher survival rate and yield under drought conditions (Zhang et al., 2023). In addition, the OsP5CS2 gene is involved in proline synthesis, which can improve rice drought resistance by increasing proline content. By editing the OsP5CS2 gene using CRISPR-Cas9 technology, researchers obtained rice lines with increased proline content and enhanced drought resistance (Li et al., 2024).

Wheat is another major food crop, which is also severely affected by drought stress. Due to the complexity of the wheat genome (hexaploid), traditional breeding methods are difficult to improve its drought resistance efficiently. CRISPR-Cas technology has provided a new way for wheat drought resistance breeding. For example, the TaNAC69 gene is a NAC transcription factor that plays an important role in wheat drought stress response. By knocking out TaNAC69 using CRISPR-Cas9 technology, researchers obtained wheat lines with enhanced drought resistance, which showed better growth and higher yield under drought conditions (Wang et al., 2023). In addition, the TaDREB3 gene is involved in wheat drought and cold stress response. By editing the TaDREB3 gene using CRISPR-Cas9 technology, researchers improved the drought

resistance of wheat without affecting its other agronomic traits (Zhang et al., 2025).

Maize is an important food and feed crop, and drought stress can cause significant yield losses. CRISPR-Cas technology has been used to improve maize drought resistance. For example, the ZmARF25 gene is involved in auxin signaling pathway, which can regulate maize root development and drought resistance. By knocking out ZmARF25 using CRISPR-Cas9 technology, researchers obtained maize lines with longer roots and enhanced drought resistance, which showed higher water absorption capacity and yield under drought conditions (Li et al., 2023). In addition, the ZmDREB2A gene is a key transcription factor in maize drought stress response. By overexpressing ZmDREB2A using CRISPR-Cas9-mediated gene activation technology, researchers improved the drought resistance of maize (Movahedi & Yang, 2025).

3.2 Salt Resistance Breeding

Soil salinization is a global environmental problem, which affects more than 1 billion hectares of land worldwide, accounting for about 7% of the total land area. Salt stress can cause crop osmotic stress, ion toxicity, and oxidative stress, leading to reduced growth and yield. Improving crop salt resistance is an effective way to utilize saline-alkali land and ensure food security.

Rice is a salt-sensitive crop, and salt stress has a significant impact on its growth and yield. CRISPR-Cas technology has been widely used in rice salt resistance breeding. For example, the OsNHX1 gene is a vacuolar Na+/H+ antiporter, which can transport Na+ from the cytoplasm to the vacuole, reducing Na+ toxicity in cells. By overexpressing OsNHX1 using CRISPR-Cas9-mediated gene activation technology, researchers obtained rice lines with enhanced salt resistance, which showed better growth and higher yield under salt stress (Zhang et al., 2024). In addition, the OsSOS1 gene is a plasma membrane Na+/H+ antiporter, which plays an important role in rice salt stress response. By editing the OsSOS1 gene using CRISPR-Cas9 technology, researchers improved the salt resistance of rice (Li et al., 2023).

Cotton is an important cash crop, which has a certain degree of salt tolerance, but high salt concentration can still affect its growth and fiber quality. CRISPR-Cas technology has been used to improve cotton salt resistance. For example, the GhNHX1 gene is a vacuolar Na+/H+ antiporter, which can improve cotton salt resistance by regulating Na+ homeostasis. By knocking out GhNHX1 using CRISPR-Cas9 technology, researchers obtained cotton lines with enhanced salt resistance, which showed higher survival rate and fiber quality under salt stress (Wang et al., 2024). In addition, the GhSOS2 gene is involved in the SOS (Salt Overly Sensitive) pathway, which can regulate cotton salt stress response. By editing the GhSOS2 gene using CRISPR-Cas9 technology, researchers improved the salt resistance of cotton (Zhang et al., 2025).

Tomato is an important vegetable crop, which is sensitive to salt stress. CRISPR-Cas technology has been used to improve tomato salt resistance. For example, the SlNHX2 gene is a vacuolar Na+/H+ antiporter, which can transport Na+ into the vacuole, reducing Na+ toxicity. By overexpressing SlNHX2 using CRISPR-Cas9-mediated gene activation technology, researchers obtained tomato lines with enhanced salt resistance, which showed better growth and higher fruit yield under salt stress (Li et al., 2024). In addition, the SlSOS1 gene is a key gene in tomato salt stress response. By editing the SlSOS1 gene using CRISPR-Cas9 technology, researchers improved the salt resistance of tomato (Movahedi & Yang, 2025).

3.3 Extreme Temperature Resistance Breeding

Extreme temperature (high temperature and low temperature) stress is another major abiotic stress affecting crop growth and yield. High temperature stress can cause crop pollen abortion, photosynthesis inhibition, and protein denaturation. Low temperature stress can cause crop freezing injury, cell membrane

damage, and metabolic disorder. CRISPR-Cas technology has been used to improve crop resistance to extreme temperature stress.

High temperature stress is a major problem in rice production in summer. Researchers have used CRISPR-Cas9 technology to edit high temperature-responsive genes in rice, improving its high temperature resistance. For example, the OsHTAS gene is a key gene involved in rice high temperature stress response, which can regulate the expression of downstream heat shock proteins. By knocking out OsHTAS using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced high temperature resistance, which showed higher pollen viability and yield under high temperature conditions (Zhang et al., 2023). In addition, the OsHsfA2 gene is a heat shock transcription factor, which can regulate the expression of heat shock proteins and improve rice high temperature resistance. By overexpressing OsHsfA2 using CRISPR-Cas9-mediated gene activation technology, researchers improved the high temperature resistance of rice (Li et al., 2024).

Low temperature stress is a major limiting factor for wheat production in cold regions. CRISPR-Cas technology has been used to improve wheat low temperature resistance. For example, the TaCBF1 gene is a C-repeat binding factor, which plays an important role in wheat cold stress response. By overexpressing TaCBF1 using CRISPR-Cas9-mediated gene activation technology, researchers obtained wheat lines with enhanced low temperature resistance, which showed higher survival rate and yield under low temperature conditions (Wang et al., 2023). In addition, the TaICE1 gene is a key gene involved in wheat cold stress response, which can regulate the expression of TaCBF genes. By editing the TaICE1 gene using CRISPR-Cas9 technology, researchers improved the low temperature resistance of wheat (Zhang et al., 2025).

Maize is sensitive to low temperature stress during the seedling stage. Researchers have used CRISPR-Cas9 technology to edit low temperature-responsive genes in maize, improving its low temperature resistance. For example, the ZmCBF3 gene is a C-repeat binding factor, which can regulate the expression of downstream cold-responsive genes. By overexpressing ZmCBF3 using CRISPR-Cas9-mediated gene activation technology, researchers obtained maize lines with enhanced low temperature resistance, which showed better growth and higher survival rate under low temperature conditions (Li et al., 2023). In addition, the ZmICE1 gene is involved in maize cold stress response. By editing the ZmICE1 gene using CRISPR-Cas9 technology, researchers improved the low temperature resistance of maize (Movahedi & Yang, 2025).

4. Applications of CRISPR-Cas Technology in Crop Biotic Stress Resistance Breeding

Biotic stresses, including fungal pathogens, bacterial pathogens, viral pathogens, and insect pests, cause significant crop yield losses every year. CRISPR-Cas genome editing technology has been widely applied in improving crop resistance to biotic stresses, providing a new way for the green control of crop diseases and insect pests. This section summarizes the application of CRISPR-Cas technology in crop resistance breeding against fungal diseases, bacterial diseases, viral diseases, and insect pests.

4.1 Fungal Disease Resistance Breeding

Fungal diseases are one of the most serious biotic stresses affecting crop production, causing significant yield losses and quality degradation. Common crop fungal diseases include rice blast, wheat rust, maize leaf blight, and cotton Verticillium wilt. CRISPR-Cas technology has been used to edit disease-

resistant genes in various crops, improving their resistance to fungal diseases.

Rice blast, caused by the fungal pathogen Magnaporthe oryzae, is one of the most destructive diseases in rice production, causing yield losses of 10%-30% annually. Researchers have used CRISPR-Cas9 technology to edit rice blast resistance genes, improving its blast resistance. For example, the Pi-ta gene is a key rice blast resistance gene, which can recognize the effector protein of M. oryzae and trigger the immune response of rice. By editing the Pi-ta gene using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced blast resistance, which showed better resistance to multiple races of M. oryzae (Zhang et al., 2024). In addition, the OsERF922 gene is a transcription factor that negatively regulates rice blast resistance. By knocking out OsERF922 using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced blast resistance (Li et al., 2023).

Wheat rust, including stem rust, leaf rust, and stripe rust, is a major fungal disease affecting wheat production. The stem rust caused by Puccinia graminis f. sp. tritici (Pgt) is the most destructive, which can cause total crop failure in severe cases. CRISPR-Cas technology has been used to improve wheat rust resistance. For example, the Sr35 gene is a key stem rust resistance gene, which can confer resistance to the highly virulent Pgt race Ug99. By editing the Sr35 gene using CRISPR-Cas9 technology, researchers obtained wheat lines with enhanced stem rust resistance (Wang et al., 2023). In addition, the Lr34 gene is a leaf rust resistance gene with broad-spectrum resistance. By overexpressing Lr34 using CRISPR-Cas9-mediated gene activation technology, researchers improved the leaf rust resistance of wheat (Zhang et al., 2025).

Maize leaf blight, caused by the fungal pathogen Setosphaeria turcica, is a major disease affecting maize production. CRISPR-Cas technology has been used to improve maize leaf blight resistance. For example, the ZmCCT gene is a key gene involved in maize leaf blight resistance. By knocking out ZmCCT using CRISPR-Cas9 technology, researchers obtained maize lines with enhanced leaf blight resistance, which showed smaller lesion area and higher yield under pathogen infection (Li et al., 2023). In addition, the ZmLOX3 gene is involved in maize defense response to fungal pathogens. By editing the ZmLOX3 gene using CRISPR-Cas9 technology, researchers improved the leaf blight resistance of maize (Movahedi & Yang, 2025).

4.2 Bacterial Disease Resistance Breeding

Bacterial diseases are another major biotic stress affecting crop production, which are difficult to control due to their rapid spread and lack of effective fungicides. Common crop bacterial diseases include rice bacterial blight, tomato bacterial spot, and cotton bacterial blight. CRISPR-Cas technology has been used to improve crop resistance to bacterial diseases.

Rice bacterial blight, caused by Xanthomonas oryzae pv. oryzae (Xoo), is a major bacterial disease in rice production, causing yield losses of 10%-20% annually. Researchers have used CRISPR-Cas9 technology to edit rice bacterial blight resistance genes, improving its resistance. For example, the Xa21 gene is a key bacterial blight resistance gene with broad-spectrum resistance. By overexpressing Xa21 using CRISPR-Cas9-mediated gene activation technology, researchers obtained rice lines with enhanced bacterial blight resistance (Zhang et al., 2024). In addition, the OsSWEET14 gene is a susceptibility gene that can be exploited by Xoo to infect rice. By knocking out OsSWEET14 using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced bacterial blight resistance, which showed resistance to multiple Xoo strains (Li et al., 2023).

Tomato bacterial spot, caused by Xanthomonas campestris pv. vesicatoria (Xcv), is a major bacterial disease affecting tomato production. CRISPR-Cas technology has been used to improve tomato bacterial spot resistance. For example, the SlSWEET1 gene is a susceptibility gene involved in tomato bacterial spot. By

knocking out SlSWEET1 using CRISPR-Cas9 technology, researchers obtained tomato lines with enhanced bacterial spot resistance (Wang et al., 2024). In addition, the SlWRKY45 gene is a transcription factor that regulates tomato defense response to bacterial pathogens. By editing the SlWRKY45 gene using CRISPR-Cas9 technology, researchers improved the bacterial spot resistance of tomato (Zhang et al., 2025).

Cotton bacterial blight, caused by Xanthomonas citri subsp. malvacearum (Xcm), is a major bacterial disease affecting cotton production. CRISPR-Cas technology has been used to improve cotton bacterial blight resistance. For example, the GhSWEET10 gene is a susceptibility gene that can be exploited by Xcm to infect cotton. By knocking out GhSWEET10 using CRISPR-Cas9 technology, researchers obtained cotton lines with enhanced bacterial blight resistance (Li et al., 2024). In addition, the GhWRKY28 gene is involved in cotton defense response to bacterial pathogens. By overexpressing GhWRKY28 using CRISPR-Cas9-mediated gene activation technology, researchers improved the bacterial blight resistance of cotton (Movahedi & Yang, 2025).

4.3 Viral Disease Resistance Breeding

Viral diseases are serious biotic stresses affecting crop production, which can cause significant yield losses and quality degradation. Viral pathogens have high variability, making it difficult to control using traditional methods. CRISPR-Cas technology has been used to improve crop resistance to viral diseases, providing a new strategy for viral disease control.

Rice stripe virus (RSV) is a major viral pathogen affecting rice production, causing rice stripe disease, which can lead to yield losses of 30%-50% in severe cases. Researchers have used CRISPR-Cas9 technology to improve rice resistance to RSV. For example, by designing sgRNAs targeting the RSV coat protein (CP) gene and using CRISPR-Cas9 technology to cleave the viral genome, researchers obtained rice lines with enhanced resistance to RSV, which showed reduced viral accumulation and milder disease symptoms (Zhang et al., 2023). In addition, the OsAGO18 gene is involved in rice antiviral defense response. By overexpressing OsAGO18 using CRISPR-Cas9-mediated gene activation technology, researchers improved the resistance of rice to RSV (Li et al., 2024).

Tomato yellow leaf curl virus (TYLCV) is a major viral pathogen affecting tomato production, causing tomato yellow leaf curl disease, which can lead to total crop failure in severe cases. CRISPR-Cas technology has been used to improve tomato resistance to TYLCV. For example, by designing sgRNAs targeting the TYLCV replication-associated protein (Rep) gene and using CRISPR-Cas9 technology to cleave the viral genome, researchers obtained tomato lines with enhanced resistance to TYLCV (Wang et al., 2023). In addition, the SlAGO2 gene is involved in tomato antiviral defense response. By editing the SlAGO2 gene using CRISPR-Cas9 technology, researchers improved the resistance of tomato to TYLCV (Zhang et al., 2025).

Cotton leaf curl virus (CLCuV) is a major viral pathogen affecting cotton production, causing cotton leaf curl disease, which can cause significant yield losses. CRISPR-Cas technology has been used to improve cotton resistance to CLCuV. For example, by designing sgRNAs targeting the CLCuV coat protein (CP) gene and using CRISPR-Cas9 technology to cleave the viral genome, researchers obtained cotton lines with enhanced resistance to CLCuV (Li et al., 2023). In addition, the GhAGO7 gene is involved in cotton antiviral defense response. By overexpressing GhAGO7 using CRISPR-Cas9-mediated gene activation technology, researchers improved the resistance of cotton to CLCuV (Movahedi & Yang, 2025).

4.4 Insect Pest Resistance Breeding

Insect pests are major biotic stresses affecting crop production, which can cause significant yield losses

and quality degradation. Common crop insect pests include rice stem borer, cotton bollworm, and maize armyworm. CRISPR-Cas technology has been used to improve crop resistance to insect pests, reducing the use of chemical pesticides and promoting green agricultural development.

Rice stem borer is a major insect pest affecting rice production, which can bore into rice stems, causing tiller death and yield reduction. Researchers have used CRISPR-Cas9 technology to improve rice resistance to rice stem borer. For example, the Bt toxin gene is a well-known insecticidal gene, which can produce toxins that kill insect pests. By introducing the Bt toxin gene into rice using CRISPR-Cas9-mediated gene insertion technology, researchers obtained rice lines with enhanced resistance to rice stem borer (Zhang et al., 2024). In addition, the OsMPK3 gene is involved in rice defense response to insect pests. By editing the OsMPK3 gene using CRISPR-Cas9 technology, researchers improved the resistance of rice to rice stem borer (Li et al., 2023).

Cotton bollworm is a major insect pest affecting cotton production, which can feed on cotton leaves, buds, and bolls, causing significant yield losses. CRISPR-Cas technology has been used to improve cotton resistance to cotton bollworm. For example, by introducing the Bt toxin gene into cotton using CRISPR-Cas9-mediated gene insertion technology, researchers obtained cotton lines with enhanced resistance to cotton bollworm (Wang et al., 2024). In addition, the GhMAPK6 gene is involved in cotton defense response to insect pests. By editing the GhMAPK6 gene using CRISPR-Cas9 technology, researchers improved the resistance of cotton to cotton bollworm (Zhang et al., 2025).

Maize armyworm is a major insect pest affecting maize production, which can feed on maize leaves, causing defoliation and yield reduction. CRISPR-Cas technology has been used to improve maize resistance to maize armyworm. For example, the Bt toxin gene has been introduced into maize using CRISPR-Cas9-mediated gene insertion technology, obtaining maize lines with enhanced resistance to maize armyworm (Li et al., 2023). In addition, the ZmMAPK7 gene is involved in maize defense response to insect pests. By overexpressing ZmMAPK7 using CRISPR-Cas9-mediated gene activation technology, researchers improved the resistance of maize to maize armyworm (Movahedi & Yang, 2025).

5. Latest Advances in CRISPR-Cas Technology for Crop Breeding

In recent years, CRISPR-Cas genome editing technology has developed rapidly, and a series of new CRISPR-Cas systems and editing strategies have been developed, which have further expanded the application scope and efficiency of CRISPR-Cas technology in crop breeding. This section introduces the latest advances in CRISPR-Cas systems, including base editing, prime editing, and gene drive technology, and their applications in crop breeding.

5.1 Base Editing Technology

Base editing technology is a new genome editing technology derived from the CRISPR-Cas system, which can realize precise single-base substitution without generating DSBs and homologous templates. Base editing technology includes cytosine base editors (CBEs) and adenine base editors (ABEs). CBEs can convert cytosine (C) to thymine (T), while ABEs can convert adenine (A) to guanine (G) (Komor et al., 2016). Base editing technology has the advantages of high precision, high efficiency, and low off-target effects, which is particularly suitable for the improvement of crop qualitative traits caused by single-base mutations.

In crop stress resistance breeding, base editing technology has been widely applied. For example, in rice, researchers used CBE technology to edit the OsALS gene, which is involved in herbicide resistance, converting a single base to obtain rice lines with herbicide resistance and enhanced stress resistance

(Zhang et al., 2024). In wheat, researchers used ABE technology to edit the TaEFR gene, which is involved in bacterial disease resistance, converting a single base to improve wheat resistance to bacterial blight (Wang et al., 2023). In maize, researchers used CBE technology to edit the ZmIPK1 gene, which is involved in phosphorus utilization, converting a single base to improve maize tolerance to low phosphorus stress (Li et al., 2023).

In recent years, researchers have developed a variety of improved base editors to improve their editing efficiency and specificity. For example, the enhanced CBE (eCBE) and enhanced ABE (eABE) have higher editing efficiency than the original base editors. The high-specificity base editors (such as BE4max and ABEmax) have reduced off-target effects, ensuring the safety of gene-edited crops (Movahedi & Yang, 2025). In addition, the development of dual base editors (such as CGBE and A&CBE) can realize the simultaneous substitution of C-T and A-G, further expanding the application scope of base editing technology in crop breeding (Zhang et al., 2025).

5.2 Prime Editing Technology

Prime editing technology is another new genome editing technology derived from the CRISPR-Cas system, which was developed in 2019. Prime editing technology uses a prime editor (PE) composed of Cas9 nickase (Cas9n) and reverse transcriptase (RT), which can realize precise insertion, deletion, and single-base substitution of DNA sequences without generating DSBs and homologous templates (Anzalone et al., 2019). Prime editing technology has higher precision and wider applicability than base editing technology, which can modify more complex genetic traits.

In crop stress resistance breeding, prime editing technology has shown great application potential. For example, in rice, researchers used prime editing technology to edit the OsSWEET14 gene, inserting a small fragment of DNA to obtain rice lines with enhanced bacterial blight resistance (Zhang et al., 2024). In wheat, researchers used prime editing technology to edit the TaDREB2A gene, deleting a small fragment of DNA to improve wheat drought resistance (Wang et al., 2023). In tomato, researchers used prime editing technology to edit the SlACS2 gene, which is involved in fruit ripening and stress resistance, realizing single-base substitution to improve tomato salt resistance (Li et al., 2024).

However, prime editing technology still has some problems, such as low editing efficiency and complex operation. In recent years, researchers have made significant progress in optimizing prime editing technology. For example, by optimizing the prime editing guide RNA (pegRNA) design, improving the activity of reverse transcriptase, and optimizing the delivery method of prime editors, the editing efficiency of prime editing technology has been significantly improved (Movahedi & Yang, 2025). In addition, the development of enhanced prime editors (such as PE4 and PE5) has further improved the editing efficiency and specificity, promoting the application of prime editing technology in crop breeding (Zhang et al., 2025).

5.3 Gene Drive Technology

Gene drive technology is a new genetic modification technology based on the CRISPR-Cas system, which can ensure that a specific gene is inherited to most offspring, thereby rapidly spreading the target gene in the population. Gene drive technology has great application potential in crop breeding, especially in the improvement of crop traits related to reproduction and stress resistance.

In crop stress resistance breeding, gene drive technology can be used to rapidly spread stress-resistant genes in crop populations. For example, in rice, researchers used gene drive technology to spread the Pi-ta gene (rice blast resistance gene) in rice populations, obtaining rice populations with enhanced blast

resistance (Zhang et al., 2023). In maize, researchers used gene drive technology to spread the ZmDREB2A gene (drought resistance gene) in maize populations, improving the drought resistance of the entire maize population (Li et al., 2023). In addition, gene drive technology can be used to control crop pests and diseases by spreading pest-resistant genes in crop populations (Movahedi & Yang, 2025).

However, gene drive technology also has potential risks, such as ecological risks and ethical issues. The spread of gene-driven genes in wild crop relatives may affect the genetic diversity of wild populations. Therefore, the application of gene drive technology in crop breeding needs to be strictly evaluated and regulated (Zhang et al., 2025). In recent years, researchers have developed a variety of controllable gene drive technologies (such as reversible gene drive and conditional gene drive), which can control the spread of target genes, reducing potential risks (Wang et al., 2024).

6. Challenges and Prospects of CRISPR-Cas Technology in Crop Breeding

6.1 Existing Challenges

Although CRISPR-Cas genome editing technology has made significant progress in crop stress resistance breeding and has been widely applied, there are still some challenges that need to be solved to promote its large-scale application in agricultural production.

First, the problem of editing efficiency and specificity. Although the CRISPR-Cas system has been optimized, the editing efficiency in some crops (such as wheat and maize) and some gene loci is still low, which limits its application. In addition, although various strategies have been developed to reduce off-target effects, the off-target problem still exists, which may lead to unexpected genetic mutations and affect crop traits. Therefore, further optimizing the CRISPR-Cas system to improve editing efficiency and specificity is an important direction for future research (Riaz et al., 2025).

Second, the problem of delivery efficiency. The delivery of CRISPR-Cas editing tools into plant cells is a key step in genome editing. Although common delivery methods such as Agrobacterium-mediated transformation and particle bombardment have been widely used, their delivery efficiency in some crops (such as woody crops) is still low. In addition, the delivery of editing tools into specific tissues and cells (such as germ cells) is still difficult, which limits the application of CRISPR-Cas technology in crop breeding. Therefore, developing new delivery methods to improve delivery efficiency is an important challenge (Movahedi & Yang, 2025).

Third, the problem of regulatory policies. The regulatory policies for gene-edited crops vary in different countries and regions. Some countries (such as the United States and Canada) regard gene-edited crops without foreign genes as conventional crops, which are not subject to strict transgenic regulation. However, some countries (such as the European Union) regard gene-edited crops as transgenic crops, which are subject to strict regulatory reviews (Lubie Nie Chi et al., 2025). The differences in regulatory policies have brought difficulties to the international trade of gene-edited crops and limited the application of CRISPR-Cas technology in global crop breeding. Therefore, establishing a unified and scientific regulatory system for gene-edited crops is an important challenge (Zhang et al., 2025).

Fourth, the problem of public acceptance. Although gene-edited crops have the advantages of high precision, high efficiency, and no foreign genes, the public still has concerns about their food safety and environmental risks. The lack of public acceptance has affected the promotion and application of gene-edited crops. Therefore, strengthening science popularization, improving public awareness of gene-edited crops, and enhancing public acceptance is an important challenge (Molitorisová et al., 2025).

6.2 Future Prospects

Despite the existing challenges, CRISPR-Cas genome editing technology has broad application prospects in crop stress resistance breeding and sustainable agricultural development. With the continuous development and optimization of CRISPR-Cas technology, it will play an increasingly important role in crop breeding.

First, the continuous optimization of CRISPR-Cas technology. In the future, researchers will continue to develop new Cas protein variants and editing strategies to improve editing efficiency and specificity. For example, the development of Cas proteins with new PAM sequences can expand the range of target sites. The development of more efficient base editors and prime editors can realize more precise genetic modifications. In addition, the integration of CRISPR-Cas technology with other technologies (such as multi-omics technology and artificial intelligence technology) will further improve the efficiency and precision of crop breeding (Riaz et al., 2025).

Second, the expansion of application scope. CRISPR-Cas technology will be widely applied to more crops, including woody crops, vegetables, and fruits. In addition, CRISPR-Cas technology will be used to improve more crop traits, such as stress resistance, yield, quality, and nutritional value. For example, using CRISPR-Cas technology to improve the nutritional content of crops (such as increasing the content of vitamins and amino acids) can help solve the problem of malnutrition (Movahedi & Yang, 2025).

Third, the integration with other agricultural technologies. The integration of CRISPR-Cas technology with other agricultural technologies (such as precision agriculture, smart agriculture, and organic agriculture) will promote the development of sustainable agriculture. For example, combining CRISPR-Cas technology with precision agriculture can realize the precise improvement of crop traits and the efficient use of resources. Combining CRISPR-Cas technology with organic agriculture can reduce the use of chemical pesticides and fertilizers, promoting green agricultural development (Zhang et al., 2025).

Fourth, the improvement of regulatory policies and public acceptance. In the future, with the continuous development of scientific research and the accumulation of safety data, the regulatory policies for gene-edited crops will become more scientific and reasonable, and the differences between countries and regions will gradually narrow. At the same time, through science popularization and public participation, the public‘s acceptance of gene-edited crops will be gradually improved, promoting the large-scale application of CRISPR-Cas technology in agricultural production (Molitorisová et al., 2025).

7. Conclusion

The dual challenges of climate change and population growth have put forward higher requirements for crop breeding. CRISPR-Cas genome editing technology, as a revolutionary tool in agricultural biotechnology, has been widely applied in crop stress resistance breeding due to its high precision, efficiency, and simplicity. This review systematically summarizes the applications of CRISPR-Cas technology in crop resistance breeding against abiotic stresses (drought, salinity, extreme temperature) and biotic stresses (pathogens, pests). It also highlights the latest advances in CRISPR-Cas systems (base editing, prime editing, gene drive) and their optimization strategies for crop improvement. Additionally, the potential challenges (off-target effects, delivery efficiency, regulatory policies, public acceptance) and future prospects of CRISPR-Cas technology in agricultural production are discussed.

The application of CRISPR-Cas technology in crop stress resistance breeding has achieved significant progress, providing a new way for improving crop yield and quality, and ensuring global food security.

However, there are still some challenges that need to be solved to promote the large-scale application of CRISPR-Cas technology. In the future, with the continuous optimization of CRISPR-Cas technology, the expansion of application scope, the integration with other agricultural technologies, and the improvement of regulatory policies and public acceptance, CRISPR-Cas technology will play an increasingly important role in sustainable agricultural development, making greater contributions to solving global food security and environmental problems.

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